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Sexing Study of Interphase Duodenum and Liver Nuclei in the Sixteen-Day Chick Embryo

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THESIS DEFENSE

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BY

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ABSTRACT

A study was made to confirm the existence of sex chromatin or some other sexual dimorphism in the interphase nuclei of the 16-day chick embryo. A Feulgen staining procedure was performed on sectioned epithelial tissues of the intestinal villi as well as sections and squashes of liver tissues.

All chromatin masses, not just those suspected to represent sex chromatin, were recorded using a blind technique. The size, location and stain intensity of these chromatin masses were recorded, and seven different studies were made comparing male and female nuclei for sexual dimorphisms.

No sex chromatin was found that resembled either that of mammalian nuclei or that described by previous authors working with chick material. The chromatin bodies, regardless of sex, varied greatly in size and number from one nucleus to another within a tissue.

A sexual dimorphism, however, was observed. Statistical evidence showed that male nuclei have a greater number of large chromatin bodies, and a lesser number of small chromatin bodies than female nuclei.

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INTRODUCTION

(1) Sex chromatin in general

Sex chromatin, commonly called the Barr body, has proven to be of tremendous value in sex determination since its discovery in cat neurons by Murray L. Barr and Etwart G. Bertram (1949). They referred to it as the 'nucleolar satellite' in that it was more or less intimately associated with the nucleolus. Actually, the structure was first noted by Cajal (1909) using dog, cat, and human nerve cells and called the paranucleolus. It has since been referred to as X-chromatin by Moore (1966) and as the drumstick in leukocytes since it takes that appearance in attaching to the nucleus (Davidson and Smith, 1956).

In human cells, sex chromatin superficially appears as a planoconvex mass adjacent to the inner surface of the nuclear membrane (Moore, 1966, p. 17). Upon closer examination, however, the most common form is a bipartite structure in which two small masses averaging 0.5 micron in length lie close to each other separated by a distance averaging 0.3 microns (Klinger, 1958). From 20 to 96% of human female nuclei contain sex chromatin depending on the tissue and stain used and on differences in methods of counting. A comparable structure, however, is rare in human male nuclei.

The location of the sex chromatin occurs 86% of the time at the periphery of the nucleus (Miles, 1961). Levy and Meulendijk (1962), however, presented mathematical evidence that the sex chromatin masses should always lie in the inner

surface of the nuclear membrane, and that the lower percentages reported were the result of the limitation of the resolving power of the microscope.

Sex chromatin was shown by Susumu Ohno and Sajiro Makino (1961) to be a single, condensed X-chromosome folded upon itself. This X-chromosome was hypothesized to be genetically inactivated thus serving as a means of dosage compensation between the sexes (Lyon, 1961). In the studies of the sex-linked color trait of the mouse, Lyon reported that a mosaic coloration in female mice was due to the inactivation of either the maternal or paternal X-chromosome in different cells of the same animal. If the X-chromosome carrying the mutant gene is inactivated, the mouse will have a normal patch of color; but, if the X-chromosome carrying a normal gene is inactivated, then a mutant (usually lighter) patch of color arises. Thus, the animal appears mosaic with patches of both normal color and mutant color.

The X-chromosome responsible for the sex chromatin body synthesizes DNA later than the other chromosomes of the complex. This was demonstrated by Taylor (1960) using tritiated thymidine and an autoradiography technique.

Sex chromatin has developed as a reliable tool in the diagnosis of sexual abnormalities. The presence or absence of sex chromatin is used as a guide to the number of X-chromosomes in the nuclei of patients suspected of having a sex chromosome abnormality. The number of sex chromatin bodies is one less than the number of X-chromosomes in the

complement. A patient with Turner's syndrome (XO) would show no sex chromatin body. Patients with Klinefelter's syndrome (XXY, XXXY or XXXXY) would show one, two, and three sex chromatin bodies respectively. Sex chromatin tests have also been useful in antenatal sex diagnosis to prevent the birth of a male fetus carrying a sex-linked hereditary disease such as hemophilia or muscular dystrophy. The procedure of collecting amniotic fluid for such tests has been used successfully in Denmark and Sweden. Sex chromatin has also been sparingly used as a marker to follow grafts in tissue; for example, it was used to follow monocyte participation in connective tissue repair (Hulliger and Allgower, 1963).

A sex chromatin mass or a sexual dimorphism (a structural difference between the sexes) has been reported in the female nuclei of various tissues in the following species of animals: water striders, spruce budworms, silkworms and decapods among the arthropods, and in men, apes, monkeys, black bears, dogs, cats, foxes, minks, cattle, sheep, deer, horses, moles, hamsters, mice, rate, rabbits and others among the mammals (Moore, 1966). Several other investigators have also reported sex chromatin in domestic fowl.

(2) Sex chromatin in the chick

Domestic fowl have a different sex chromosome composition than mammals in that the male is homozygous ZZ and the female is heterozygous ZW. If the barr body, therefore, served as a means of dosage compensation in chickens as it does in mammals, then it should be found in male nuclei. However,

all reports of sex chromatin in interphase chick nuclei are in female rather than male nuclei.

In general, however, the presence of sex chromatin in chick nuclei and its use in sex determination is controversial. Five former investigations, cited below, report partial to full sucess in finding sex chromatin or a sexual dimorphism in interphase chick nuclei, while three other investigations, also cited below, failed to show any definite chromatin difference in relation to sex.

(a) Investigations with positive results

In the original report of the existence of sex chromatin, I. L. Kosin and Hironi Ishizaki (1959) demonstrated sex chromatin in a position closely apposed to the nuclear membrane in three-week old New Hampshire female chicks. Best results were obtained using Harris' hematoxylin on duodenal smooth muscle and dermal and epidermal cells at the base of growing feathers.

A futher study of Ishizaki and Kosin (1960) showed that a "sex dimorphism with respect to sex chromatin was clear cut" in amniotic nuclei of nine to 14-day chick embryos. 'Bi-modal' sex chromatin patterns were also obtained in five-day amniotic cells and two-day area opaca cells in the chick embryo. The sexual dimorphism in the youngest stage was supported by biopses of 16 embryos taken as controls.

The third set of positive results was obtained by S. Ohno, W. D. Kaplin, and R. Kinosita (1960). These investigators concluded from their work with 10 to 17-day white leghorn embryos that sex chromatin in the female chick represented a single Z-chromosome. Using feulgen squashes of skin and liver cells, it was observed that the nuclei of both sexes had from three to eight small chromocenters, but that "a large chromocenter of distinctly bipartite nature" measuring a little short of two micra was found in well flattened female nuclei.

Keith L. Moore and Jean C. Hay (1961) found "fairly distinct sexual dimorphism" in epidermal and smooth muscle cells of the duodenum. Accurate sexing of cells by means of nuclear chromatin patterns in epithelium of kidney, liver, duodenum and other tissues, however, proved to be a failure.

In the fifth report indicating positive results, V. Ya Azarova (1961), using a feulgen liver squash technique on 16-day embryos, showed that about 71% of female nuclei contained a sex chromatin clump measuring from 0.7 to 1.5 microns, while only about 16% of males showed a similar clump. In all, 81 to 85% of these chromatin clumps were located in the nucleoplasm.

(b) Investigations with negative results

David B. Ashley and Erich A. Theiss (1959)

reported that no sex chromatin body similar to that of human and other mammals was found in epidernal, liver, heart, epithelium and smooth muscle tissues of adult chickens. At times when a sex difference was thought to be recognizable, it was not substantiated in blind testing in which the investigator had no former knowledge of the actual sex of the cells. Whether using hematoxylin and eosin or a feulgen technique, they noted no consistent trend in the number of chromatin bodies.

In the second investigation with negative results, C. P. Miles and S. D. Storey (1962) were unable to sex cultures of heart, spleen and kidney cells using a blind technique. They also noted that the nuclear chromocenters of both male and female cells from two-week to two-month old hatched chicks "varied markedly in incidence and were commonly smaller than those occuring in mammalian cells."

In the final negative report listed here, Hammar (1964), using a feulgen squash technique on eight different adult white leghorn tissues including liver and duodenum considered the largest chromatin body near the nuclear membrane as sex chromatin. He found it in 93% of female cells and 94% of male cells. Hammar did, however,

report a significant correlation between sex and color intensity of the heterochromatic bodies with female cells showing darker chromatin bodies than male cells.

(3) Statement of the problem

With respect to the investigations cited above a clear-cut answer to the problem of interphase nuclear sexing of chick cells is still unsolved. In those investigations reporting positive findings, the descriptions of sex chromatin have not been consistent. The location as well as the size of the chromatin mass is unresolved. A report that sex chromatin was located closely apposed to the nuclear membrane was contrary to another report which located it 80 to 85% of the time in the nucleoplasm. The size of sex chromatin was noted to be just short of two micra in one report, while in another it was measured from 0.7 to 1.5 microns. Also, the color intensity was only considered as a possible factor by one investigator.

This investigation considered the existence of sex chromatin or a sexual dimorphism in the 16-day chick embryo. using liver and epithelial cells of the villi. This investigation, unlike those previously recorded, collected the data with no preconceived ideas about the location or size of the sex chromatin body. Therefore, the size, location and stain intensity of all chromatin masses in each nucleus was recorded. All data collected was analyzed using a blind technique to discover if any difference exists between the chromatin structure of male and female interphase nuclei.

MATERIALS AND METHODS

Duodenal and liver tissues were obtained from a total of 20 embryos of 16-day incubation (103°). Liver and duodenal tissue were chosen since they were used in six of eight previous investigations on sex chromatin in the chick. Sexing of the embryos was easily accomplished by gross inspection of the gonads (Appendix A, plates I and II, p. 34).

A total of 30 slides were prepared as follows:

1) Section method: Ten (five of each sex) duodenal and ten (five of each sex) liver slides were prepared from small pieces of tissue fixed three to six hours in Carnoy-Lebrun solution (Humason, 1967, p. 18), washed in 50% ethyl alcohol, then soaked for five to eight hours in a 70% ethanol-iodine solution (Humason, 1967, p. 13) to remove the mercuric chloride. The tissues were dehydrated, cleared and infiltrated with paraffin. (It is to be noted that liver showed extreme brittleness and, therefore, a maximum of one hour in absolute ethanol was used for fear of damage.)

Sections were cut at three microns, mounted, brought to water, placed in Lugol's solution for three minutes to remove excess mercuric chloride, washed in water, soaked in five percent sodium thiosulfate for three minutes to wash out the iodine of the Lugol's solution, and washed in water. The sections were then brought through a feulgen staining procedure as described by Moore (1966, pp. 103, 103) in which the slides were immersed in hot 1-N hydrochloric acid for six minutes and then stained in Barger and De Lamater's

schiff reagent for a period of one hour. A counter stain was omitted since earlier testing proved it to hamper accurate counting of chromatin masses.

2) Squash method: A three millimeter square piece of liver was squashed between two slides, fixed 1½ to 2½ hours in Carnoy's acid alcohol (Humason, 1967, p. 18), transferred to 95% ethanol, hydrated, and stained as in the section method. Ten slides (five of each sex) were prepared by this method.

All 30 slides were studied with an A/O binocular microscope at a magnification of 1000X using a green filter to assure better contrast. The chromatin structure of 100 nuclei from each slide was examined using a blind technique. This technique consisted of covering the slide markings which indicate the sex of the animal until all the slides were examined. In duodenal slides, only epithelial nuclei located on the villi and measuring between 3.5 and 10 microns in diameter were used in the counts. In liver sections and squashes, nuclei ranging from four to seven microns in diameter were used.

In every nucleus studied, each chromatin body was placed in one of the following categories:

1. Large chromatin bodies ($d \geq 0.2$ microns)
    a. Touching the nuclear membrane
    b. Near the nuclear membrane (within 0.3 microns)
    c. In the nucleoplasm
2. Small chromatin bodies ($d < 0.2$ microns)

The diameter of each large chromatin body was measured and a subjective judgement was made as to its stain intensity, classifying that body as light or dark in stain intensity.

RESULTS

In each of the three slide types (duodenal sections, liver sections and liver squashes), tissues from 10 embryos were used to make 10 slides (five from male embryos and five from female embryos). In each of the 30 slides, 100 interphase nuclei were used to gather the data concerning the chromatin masses. Therefore, a total of 500 interphase nuclei of each sex were studied from each of the three slide types, and, in all cases, the total values obtained from these 500 nuclei were used to test for significant differences between the sexes. All differences with $P < 0.05$ were considered significant.

The chick interphase nuclei studied had many chromatin masses of various sizes. The total number of chromatin masses ranged between two and 21 per nucleus with a mean of 10.6. All chromatin masses having a diameter equal to or over 0.2 microns were considered as 'large' and these ranged in number from zero to 11 per nucleus with a mean of 3.5. 'Small' chromatin bodies ($d < 0.2$ microns) ranged in number from zero to 19 per nucleus with a mean of 7.1.

All the peculiar structures or flaws seen in the nuclei such as shadows, membrane folds and smudges were recorded. No 'bipartite' structures were found to exist in the nuclei studied.

Seven Studies (I-VII) were employed to test for significant differences between the sexes in the chromatin structure of the interphase nuclei.

STUDY I Number of large chromatin bodies

Study I is divided into five parts (A-E), according to location of the chromatin bodies. Only chromatin bodies with a diameter equal to or over 0.2 microns were considered as large.

A. Touching the nuclear membrane

Only large chromatin masses in direct contact with the nuclear membrane were considered in these counts. The data collected is recorded in Table IA.

Table IA. Number of large chromatin bodies touching the nuclear membrane.

	Males			Females			
	A	B	C	D	E	F	G
Slide type	Range and average no. of chromatin bodies per nucleus	Average no. of chromatin bodies per slide (100 nuclei)	Total no. of chromatin bodies for 5 slides (500 nuclei)	Range and average no. of chromatin bodies per nucleus	Average No. of chromatin bodies per slide (100 nuclei)	Total no. of chromatin bodies for 5 slides (500 nuclei)	P values obtained by chi-square test between columns C and F
Duodenal Sections	0-4(1.05)	105.2	526	0-5(1.01)	101.4	507	$P \geq 0.5$
Liver Sections	0-4(0.56)	56.2	281	0-7(0.61)	61.0	305	$P \geq 0.1$
Liver Squashes	0-4(0.56)	56.4	282	0-3(0.47)	46.6	233	$P < 0.05$*

Reference: Appendix B, Table IA, p. 40
*A significant difference

The male nuclei of the liver squash cells have significantly more large chromatin bodies touching the nuclear membrane than the female nuclei. This significant difference, however, is neither found in duodenal nor liver sections.

RESULTS

In each of the three slide types (duodenal sections, liver sections and liver squashes), tissues from 10 embryos were used to make 10 slides (five from male embryos and five from female embryos). In each of the 30 slides, 100 interphase nuclei were used to gather the data concerning the chromatin masses. Therefore, a total of 500 interphase nuclei of each sex were studied from each of the three slide types, and, in all cases, the total values obtained from these 500 nuclei were used to test for significant differences between the sexes. All differences with $P < 0.05$ were considered significant.

The chick interphase nuclei studied had many chromatin masses of various sizes. The total number of chromatin masses ranged between two and 21 per nucleus with a mean of 10.6. All chromatin masses having a diameter equal to or over 0.2 microns were considered as 'large' and these ranged in number from zero to 11 per nucleus with a mean of 3.5. 'Small' chromatin bodies ($d < 0.2$ microns) ranged in number from zero to 19 per nucleus with a mean of 7.1.

All the peculiar structures or flaws seen in the nuclei such as shadows, membrane folds and smudges were recorded. No 'bipartite' structures were found to exist in the nuclei studied.

Seven Studies (I-VII) were employed to test for significant differences between the sexes in the chromatin structure of the interphase nuclei.

B. Near the nuclear membrane

Only large chromatin bodies that were not touching but were within a distance of 0.3 microns from the nuclear membrane were considered. The data collected is recorded in Table IB.

Table IB. Number of large chromatin bodies located near (within 0.3 microns) the nuclear membrane.

	Males			Females			
	A	B	C	D	E	F	G
Slide type	Range and ave. # of chromatin bodies per nucleus	Ave. # of chromatin bodies per slide (100 nuclei)	Total # of chromatin bodies for 5 slides (500 nuclei)	Range and ave. # of chromatin bodies per nucleus	Ave. # of chromatin bodies per slide (100 nuclei)	Total # of chromatin bodies for 5 slides (500 nuclei)	P values obtained by chi-square test between columns C and F
Duodenal Sections	0-4(0.45)	44.8	224	0-3(0.36)	35.6	178	$P < 0.025$*
Liver Sections	0-3(0.45)	45.4	227	0-3(0.36)	35.8	179	$P < 0.025$*
Liver Squashes	0-3(0.32)	32.4	162	0-3(0.31)	30.6	153	$P > 0.1$

Reference: Appendix B, Table IB, p. 40
*A significant difference

The nuclei of male duodenal and liver sectioned tissues showed significantly more chromatin bodies in this position than those of the female tissues. This difference was not significant in the nuclei of the liver squashes.

C. Touching and near the nuclear membrane

A combination study of the number of large chromatin masses either touching or near the nuclear membrane serves to compare the results of this report with those of I. L. Kosin ahd Hironori

Ishizaki (1959) who found sex chromatin "closely apposed to the nuclear membrane."

The data collected is recorded in Table IC.

Table IC. Combined number of large chromatin bodies touching and near the nuclear membrane. (Summary of Tables IA and IB.)

Slide type	Males A Total number of chromatin bodies for 5 slides (500 nuclei)	Females B Total number of chromatin bodies for 5 slides (500 nuclei)	P value obtained from chi-square test between columns A and B
Duodenal Sections	750	685	$P \geq 0.05$
Liver Sections	508	484	$P > 0.1$
Liver Squashes	444	386	$P < 0.05$*

Reference: Appendix B, Table IC, p. 41
*A significant difference

It was found that only male liver squash nuclei had a significantly greater number of chromatin masses touching and near the nuclear membrane. This significance, however, was chiefly due to the significantly greater number of chromatin bodies found touching the membrane (as seen in Table IA, p. 12). It was also noted that the significantly greater number of male chromatin bodies found near the membrane ($P < 0.025$) in duodenal and liver sections (refer to Table IB, p. 13) was lost due to the number of the large chromatin bodies touching the nuclear membrane in this combination study.

D. Located in the nucleoplasm

Only large chromatin masses located beyond 0.3 microns from the nuclear membrane in the nucleoplasm were considered in this study. The data collected is recorded in Table ID.

Table ID. Number of large chromatin bodies located in the nucleoplasm.

	Males			Females			
	A	B	C	D	E	F	G
	Range & av. no. of chromatin bodies per nucleus	Av. # of chromatin bodies per slide (100 nuclei)	Total # of chromatin bodies for 5 slides (500 nuclei)	Range & av. no. of chromatin bodies per nucleus	Av. no. of chromatin bodies per slide (100 nuclei)	Total # of chromatin bodies for 5 slides (500 nuclei)	P values obtained from chi-square tests between columns C&F
Duodenal Sections	0-6(2.4)	241.4	1207	0-5(197)	197.2	986	P<.005*
Liver Sections	0-7(3.1)	311.4	1557	0-8(2.74)	273.6	1368	P<.005*
Liver Squashes	0-7(2.2)	221.0	1105	0-8(2.15)	215.2	1076	P>0.5

Reference: Appendix B, Table ID, p. 41
*A significant difference

In both duodenal and liver sections, males showed significantly more large chromatin located in the nucleoplasm than females ($P \leq 0.005$). Liver squashes, however, showed no significant difference between the sexes.

E. All large chromatin bodies.

All large chromatin bodies found anywhere in the nucleus were used in this study. Combining the data of parts A, B and D into this study would prove valuable if sex chromatin had no specific location in the chick nuclei as it has in mammalian

nuclei. The data collected is recorded in Table IE.

Table IE. Total number of all large chromatin bodies.

Slide type	Males A	Females B	
	Total number of chromatin bodies for 5 male slides (500 nuclei)	Total number of chromatin bodies for 5 female slides (500 nuclei)	P values obtained from chi-square tests between columns A and B
Duodenal sections	1957	1671	$P < 0.005$*
Liver sections	2065	1852	$P < 0.005$*
Liver squashes	1549	1562	$P > 0.1$

Reference: Appendix B, Table IE, p. 42
*A significant difference

Males had significantly more large chromatin masses than females in both duodenal and liver sections. This difference was chiefly due to the significantly greater number of large chromatin bodies located in the nucleoplasm (refer to Table ID on page 15). Liver squash nuclei, on the other hand, showed only a slight but not significant difference between the sexes.

STUDY II Average sizes of the large chromatin bodies

Study II is divided into three parts based on the location of the large chromatin bodies ($d \leq 0.2$ microns).

A. Both touching and near the nuclear membrane

The average sizes of the large chromatin masses touching and near (within 0.3 microns) the

nuclear membrane were tested for differences between the sexes. The data for this study is located in Table IIA.

Table IIA. Average sizes of the large chromatin bodies touching and near the nuclear membrane.

Slide type	Males A	Females B	
	Average size in males (in microns)	Average size in females (in microns)	P values obtained from unpaired t-tests of raw data
Duodenal sections	.338	.346	$P \geq 0.9$
Liver sections	.273	.251	$P \geq 0.9$
Liver squashes	.254	.238	$P \geq 0.9$

Reference: Appendix B, Table IIA, p. 42

The data from Table IIA, as well as graphs IA, B and C of Appendix C on page 48, indicated that no appreciable difference between males and females existed in the size of the chromatin bodies touching and near the nuclear membrane.

B. Located in the nucleoplasm

The average sizes of the large chromatin bodies located further than 0.3 microns from the nuclear membrane in the nucleoplasm were tabulated in this study. The data is recorded in Table IIB.

Table IIB. Average size of the large chromatin bodies located in the nucleoplasm.

Slide type	Males A Average size in males (in microns)	Females B Average size in females (in microns)	P values obtained from unpaired t-tests of raw data
Duodenal sections	.362	.366	$P \geq 0.9$
Liver sections	.290	.286	$P \geq 0.9$
Liver squashes	.266	.254	$P \geq 0.5$

Reference: Appendix B, Table IIB, p. 43

The data from Table IIB, as well as graphs IIA, B and C of Appendix C on page 49, indicated that no significant difference between males and females existed in the average size of the chromatin bodies located in the nucleoplasm.

C. All large chromatin bodies in the nucleus

A study of the average size of all large chromatin bodies serves to combine the data of studies IIA and B. This study does seem appropriate if a large sex chromatin mass was to be found with no specific location in the female nuclei. The data of this study is recorded in Table IIC.

Table IIC. Average size of all the large chromatin bodies in nucleus.

Slide type	Males A	Females B	
	Average size in males (in microns)	Average size in females (in microns)	P values obtained from unpaired t-tests of raw data
Duodenal sections	.360	.357	$P > 0.9$
Liver sections	.290	.282	$P > 0.5$
Liver squashes	.269	.251	$P > 0.2$

Reference: Appendix B, Table IIC, p. 43

No significance was found between male and female nuclei in the average size of the total number of large chromatin bodies. This finding was also illustrated in Appendix C, graphs IIIA, B and C on page 50.

STUDY III Number of massive chromatin bodies ($d \geq 0.7$ microns). Sections A, B and C were intended to test the contention of V. Ya Azarova (1961) that sex chromatin existed in the female chick nuclei and ranged in size between 0.7 and 1.5 microns.

A. Touching and near the nuclear membrane

This part is concerned with testing for a difference between the sexes in the number of massive chromatin bodies ($d \geq 0.7$ microns) touching and near the nuclear membrane. The data of this study is contained in Table IIIA.

Table IIIA. The number of massive chromatin bodies touching and near the nuclear membrane.

Slide type	Males A Total no. found in the 5 male slides (500 nuclei)	Female B Total no. found in the 5 female slides (500 nuclei)	P values obtained from chi-square tests between columns A & B
Duodenal sections	48	39	$P \geq 0.1$
Liver sections	14	8	$P \geq 0.1$
Liver squashes	5	6	$P \geq 0.5$

Reference: Appendix B, Table IIIA, p. 44

From Table IIIA, it was noted that of a total of 3,000 nuclei studied, only 120 massive chromatin bodies were touching or near the nuclear membrane. Therefore, only four percent of the nuclei showed massive chromatin bodies at these locations. No significant differences between the sexes were noted in any of the three slide types.

B. In the nucleoplasm

This part is concerned with only the number of massive chromatin bodies ($d \leq 0.7$ microns) located further than 0.3 microns from the nuclear membrane in the nucleoplasm. The data for this study is contained in Table IIIB.

Table IIIB. Number of massive chromatin bodies located in the nucleoplasm.

Slide Type	Males A Total no. found in the 5 male slides (500 nuclei)	Females B Total no. found in the 5 female slides (500 nuclei)	P values obtained from chi-square tests between columns A & B
Duodenal sections	71	78	$P \geq 0.5$
Liver sections	44	52	$P \geq 0.1$
Liver squashes	14	22	$P \geq 0.1$

Reference: Appendix B, Table IIIB, p. 44

Table IIIB indicated that of the 3,000 nuclei studied, only 281 massive chromatin bodies were found in the nucleoplasm. Therefore, only nine percent of the nuclei had massive chromatin bodies in this position. No appreciable difference between the sexes was evident in any of the slide types tested.

C. All massive chromatin bodies of the nucleus.

This part tests for a sex difference in all the massive chromatin bodies regardless of the location. It represents a totaling of parts A and B and would be necessary if the sex chromatin body reported by V. Ya Azarova (1961) had no specific location in the nucleus. The data for this study is contained in Table IIIC.

Table IIIC. Total number of all massive chromatin bodies of the nucleus.

Slide Type	Males A Total no. found in the 5 male slides (500 nuclei)	Females B Total no. found in the 5 female slides (500 nuclei)	P values obtained from chi-square tests between columns A & B
Duodenal sections	119	117	$P \geq 0.9$
Liver sections	58	60	$P \geq 0.5$
Liver squashes	19	28	$P \geq 0.1$

Reference: Appendix B, Table IIIC, p. 45

Table IIIC indicates that of the 3,000 nuclei studied, a total of only 401 massive chromatin bodies were found regardless of their location. Therefore, only 13% of the nuclei studied had massive chromatin bodies. In all three slide types, no significant differences between the sexes was found. Female liver squash nuclei, however, did show more massive chromatin bodies than male nuclei, but the probability level ($P \geq 0.1$) was not significant.

STUDY IV. Number of small chromatin bodies

The purpose of this study was to test for a difference between the sexes in the total number of small chromatin bodies regardless of location in the nuclei. Included in this study were the smallest chromatin masses to be resolved, those less than 0.2 microns. The data for this study is found in Table IV.

Table IV. Number of all small chromatin bodies of the nucleus.

	Males			Females			
	A	B	C	D	E	F	G
Slide Type	Range and average no. of chromatin bodies per nucleus	Average no. of chromatin bodies per slide (100 nuclei)	Total no. of chromatin bodies for 5 slides (500 nuclei)	Range and average no. of chromatin bodies per nucleus	Average no. of chromatin bodies per slide (500 nuclei)	Total no. of chromatin bodies for 5 slides (500 nuclei)	P values obtained by chi-square tests between columns C and F
Duodenal sections	0-15(4.95)	495.2	2476	1-14(5.53)	553.4	2767	P<0.005*
Liver sections	2-14(7.04)	703.8	3519	2-19(7.55)	755.2	3776	P<0.005*
Liver squashes	3-16(7.75)	775.0	3875	3-18(9.85)	984.6	4923	P<0.005*

Reference: Appendix B, Table IV, p. 45
*A significant difference

From Table IV, it is observed that a total of 21,336 small chromatin bodies were found in the 3,000 nuclei which averages slightly more than seven per nucleus. In all three slide types, female nuclei had a significantly greater number of these small chromatin bodies than did male nuclei.

STUDY V. Total number of all chromatin bodies of the nucleus

Study V is a combination study of all chromatin bodies whether large or small regardless of the location. Separating large and small chromatin bodies at a diameter of 0.2 microns was an arbitrary point chosen in this investigation. It would be interesting to test for the possibility of one sex having a greater number of chromatin bodies than

the other. The data for this study is contained in Table V.

Table V. Total number of all chromatin bodies.

Slide Type	Males A	Females B	
	Total no. found in the 5 male slides (500 nuclei)	Total no. found in the 5 female slides (500 nuclei)	P values obtained from chi-square tests between columns A & B
Duodenal sections	4433	4438	$P > 0.9$
Liver sections	5584	5628	$P > 0.5$
Liver squashes	5424	6385	$P < 0.005$*

Reference: Appendix B, Table V, p. 46
*A significant difference

In the total number of all chromatin bodies, both section slide types showed extremely close totals. In liver squashes, however, a significantly greater number of chromatin bodies were found in female nuclei. This finding was completely due to the greater number of small chromatin masses found in these nuclei, as seen in Table IV on p. 23.

STUDY VI. Frequency of location for the largest chromatin body.

Study VI considers differences between the sexes in the location of the largest chromatin body. If sex chromatin existed in the chick and if it were similar to that of mammalian nuclei, a significantly greater percentage of the largest chromatin body in females should be touching or near the nuclear membrane rather than in the nucleoplasm. The data for this

study is contained in Table VI.

Table VI. Percent where the largest chromatin body is located.

Slide Type	Location	Average percent in males (for 100 nuclei)	Average percent in females (for 100 nuclei)	P values obtained by using chi-square tests on total percent tabulation in raw data
Duodenal sections	on and near the membrane	32.94	38.58	P>0.1
	in the nucleoplasm	67.06	61.42	
Liver sections	on and near the membrane	18.44	19.86	P>0.5
	in the nucleoplasm	81.56	80.14	
Liver squashes	on and near the membrane	25.68	25.06	P>0.975
	in the nucleoplasm	74.32	74.94	

Reference: Appendix B, Table VI, p. 46

Table VI, contrary to what was expected, showed that no significant differences exist between the sexes regardless of tissue type.

STUDY VII. Stain intensity of the large chromatin bodies

In Study VII, the percent of lightly stained chromatin bodies was calculated in each slide to test for the intensity of stain in the large chromatin bodies ($d \geq 0.2$ microns). All large chromatin bodies were considered in the count regardless of their location. The data collected is recorded in Table VII.

Table VII. Average percent of large chromatin bodies per slide classified as lightly stained.

Slide Type	Males A	Females B	
	Average percent of light chromatin bodies per slide	Average percent of light chromatin bodies per slide	P values obtained from chi-square tests of the grand total percent
Duodenal sections	17.88	14.54	$P > 0.1$
Liver sections	9.06	9.90	$P > 0.5$
Liver squashes	19.28	18.39	$P > 0.5$

Reference: Appendix B, Table VII, p. 47

Table VII illustrates that no significant differences were found between the sexes in relation to stain intensity of large chromatin bodies.

DISCUSSION

The intent of this investigation was to resolve the controversy as to the existence of sex chromatin in female chick nuclei. The relevance of this report is attributed to the method and amount of data collected. All chromatin masses, not just those suspected to represent sex chromatin, were recorded using a blind technique. After all the data was compiled, significance testing was done to compare the chromatin structure of male nuclei with that of female nuclei. If a sex chromatin mass was present in the female chick nuclei studied, it should be evident in one of the seven studies performed.

STUDY I

Study I was employed to test for any differences between the sexes in the number of large ($d \geq 0.2$ microns) chromatin bodies. If a large sex chromatin body existed in the nuclei of the female chick, it would undoubtedly be evident in this data. No chromatin body representing the sex chromatin of previous authors, however, seemed apparent in Study I.

I. L. Kosin and Hironori Ishizaki (1959) reported the sex chromatin "closely apposed to the nuclear membrane." The data of Tables IA, IB and IC on pages 12, 13 and 14 could not substantiate these findings. Study IA, concerned with the number of large chromatin touching the nuclear membrane, showed male liver squash nuclei to have a significantly greater number of chromatin masses than female ($P < 0.05$). On the other hand, both sectioned slide types showed no difference between

the sexes.

Study IB, concerned with the number of large chromatin masses located near the nuclear membrane, showed males in both duodenal and liver sections to have a significantly larger number of chromatin masses than females. Liver squash nuclei, however, had no appreciable difference between the sexes.

In the combination study of large chromatin bodies touching and near the nuclear membrane, significant differences between the sexes were found only in liver squashes with males again showing a higher number. Sectioned duodenum and sectioned liver showed no appreciable difference between the sexes.

In all cases when significant difference between the sexes was found in the number of large chromatin bodies touching and near the nuclear membrane, males rather than females showed higher counts. These findings were the reverse of what would be expected from the results of I. L. Kosin and Hironori Ishizaki (1959) in that they indicated that there is no sex chromatin body touching or near the nuclear membrane.

Study ID, concerned with testing the number of large chromatin bodies located in the nucleoplasm, showed no differences between the sexes in liver squash nuclei. V. Ya Azarova (1961), however, using the same technique and tissue, found over 80% of the sex chromatin bodies in the nucleoplasm. In sectioned duodenal epithelium and liver cells, males showed a significantly greater number of large chromatin bodies in the nucleoplasm than females ($P \leq 0.005$). These findings were

again the reverse of what was expected from the previous investigation of V. Ya Azarova (1961) and indicated that there was no sex chromatin mass located in the nucleoplasm.

In Study IE, the combination study of all the large chromatin bodies regardless of their location, both duodenal and liver sectioned male nuclei showed a significantly higher total number of large chromatin bodies than female nuclei. Liver squash nuclei, however, showed no difference between the sexes.

From the findings in Study I, it can be said that no chromatin body representing the sex chromatin body of previous authors was found. This contention is supported by the investigation of W. Schmid (1962) in which it was demonstrated that the female Z chromosome (that chromosome contended by S. Ohno, W. D. Kaplan and R. Kinosita (1960) to be represented in interphase by the sex chromatin) behaved similar to the two Z chromosomes of the male. Therefore, neither is there late replication of the Z chromosome in female chick nuclei nor is there a dosage compensation mechanism like that found in mammalian nuclei. If sex chromatin exists at all in the nuclei of the domestic chicken, it does not serve the same purpose that sex chromatin serves in mammalian nuclei.

It was also noted that, in general, male nuclei have more large chromatin bodies ($d \geq 0.2$ microns) than female nuclei. In support of these findings, it can be said that if chromatin bodies in interphase nuclei do represent heterochromatic portions of actual chromosomes as they are thought to

(DeWitt, 1962, p. 95), then males should show more large chromatin masses than females in that males have an extra large Z chromosome in their complements. Females, on the other hand, have a small W chromosome in their complements.

Another definite trend was conspicuous in the data of Study I. In every instance, when a significant difference between the sexes was noted in the chromatin structure of sectioned tissues, it was not found in squash tissues. When a significant difference between the sexes was reported in the liver squash nuclei, however, it was not found in sectioned material. This undoubtedly illustrates the significant role that technique plays in the appearance of various chromatin bodies. In fact, it illustrates that technique is more of a factor than the tissue type used. It also could be the reason why certain former investigations had reported positive results and others using the same tissues had reported negative results.

STUDY II

Study II was concerned with the average sizes of the large chromatin bodies. In all instances, no significant differences between the sexes were noted in respect to average sizes (refer to Tables IIA, IIB and IIC on pages 17, 18 and 19). It can be noted, however, that there was a trend for males to show a slightly greater average size in their large chromatin bodies than females. This data was perhaps also contrary to what is expected if a large sex chromatin clump was to be found in female nuclei. It, however, does seem logical if no such

clump exists, since males, having an extra large Z chromosome, should have, on the average, larger chromatin masses at interphase. Again, this presupposes that chromatin masses do represent heterochromatic portions of specific chromosomes.

STUDY III

Study III tested for differences between the sexes in the number of massive chromatin bodies (d=0.7 microns). This study was used specifically to try to corroborate the data of V. Ya Azarova (1961) and S. Ohno, W. D. Kaplan, and R. Kinosita (1960). In no instance was a significant difference found between the sexes, regardless of the location tested or the tissue type used.

In no case was any structure noted that could compare in size to the 'bipartite' structure of S. Ohno, W. D. Kaplan and R. Kinosita (1960) measuring just short of two micra. The largest chromatin body recorded in this study was about one micron in diameter (refer to Appendix C on page 48).

The data of Study III did not confirm the findings of V. Ya Azarova (1961) that noted 71.48% of females to have a chromatin body between 0.7 and 1.5 microns and only 15.52% of males to have such a structure. The liver squash data presented in this report in Table IIIC on page 22 indicated that only 5.6% of the females and 3.8% of the males had a chromatin structure equal to or greater than 0.7 microns in diameter. Differences between the two reports could possibly be due to subspecies differences or differences in various technical factors such as staining procedures, thickness of sections, or measuring devices used.

STUDY IV

In Study IV, females in all three tissue types had significantly more small chromatin bodies ($d \leq 0.2$ microns) than males (refer to Table IV on page 23). This finding was expected in that females have an extra small chromosome (the W chromosome) in their complement.

Nevertheless, female liver squash had 1,048 more small chromatin masses than did males. This represents an average of greater than two small chromatin bodies per nucleus more in females than in males. This result could possibly indicate a greater stain intensity in female nuclei, thus allowing more small chromatin bodies to be resolved. If this hypothesis was true, it lends support to the investigation of Hammar (1964) in which it was noted using liver and spleen squashes that female chromatin masses were stained darker than male chromatin masses ($P \leq 0.05$).

STUDY V

Study V measured the difference between the sexes in the total number of chromatin bodies, regardless of size and location. Since the number of chromosomes is the same in male and female fowl, the two sexes should show a similar total number of chromatin bodies. In fact, duodenal sections with a count of some 8,871 chromatin bodies for both sexes showed a difference of only five chromatin bodies between males and females. In liver sections with a total count of 11,212 chromatin masses for both sexes, the difference noted was only 44 chromatin bodies (refer to Table V on page 24).

Liver squash female nuclei, however, had a significantly higher total number of chromatin bodies than did males ($P \leq 0.005$). The discrepancy between the squash nuclei and sectioned nuclei was completely due to the greater number of small chromatin bodies of female nuclei in the liver squash preparation (refer to Table IV on page 23).

STUDY VI

Study VI was performed to see if a significant difference between the sexes existed in the location of the largest chromatin body. If the data was to corroborate that of I. L. Kosin and Hironori Ishizaki (1959), it would be expected to show a significantly greater number of the largest chromatin bodies of the female touching and near the nuclear membrane. The significance testing done, however, showed no appreciable difference between the sexes. The data of this investigation did show a greater trend in females to have their largest chromatin body touching or near the nuclear membrane in both duodenal and liver sectioned nuclei (refer to Table VI on page 25). This trend, however, was reversed in liver squashed nuclei.

STUDY VII

Study VII was performed to test the contention of Hammar (1964) that "in birds the heterochromatic bodies of the male nuclei appeared to be more faintly stained than those of the female..." Contrary to Hammar (1964), the data contained in Table VII on page 26 showed that no significant difference exists between the sexes in the stain intensity of the large

chromatin bodies.

It should be made clear that in this investigation there are reservations about the method employed to collect the data for the study of stain intensity differences between the sexes. A subjective decision was made by the investigator in each instance of whether to classify the chromatin body lightly or darkly stained.

In an effort to locate the 'bipartite' structures consisting "of a pair or rods measuring a little short of two micra in length," note was taken of all odd structures, smudges, shadows and the like located in the nuclei. None of the 'bipartite' structures described by S. Ohno, W. D. Kaplan and R. Kinosita (1960) were found.

In general, the data of this investigation supports that of David J. B. Ashley and Erich A. Theiss (1959) in that "the total number of chromatin bodies was inconsistent in both sexes" and a wide range was similar in both males and females. The number of chromatin bodies ranged from a low count of two to a high count of 21 per nucleus.

This data has also collaborated that of C. P. Miles and S. C. Storey (1962) in that the nuclear chromatin bodies were noted to be commonly smaller than those in mammalian nuclei.

It was concluded from the results of the seven studies that sex chromatin is not recognizable in the interphase nuclei of the 16-day chick embryo using a blind technique on sectioned duodenal epithelium cells of the villi, sectioned liver cells and squash liver cells. No significant

differences were found between the sexes in the average size of the large chromatin bodies, the number of massive chromatin bodies, the location of the largest chromatin body, and the stain intensity of the large chromatin bodies.

A sexual dimorphism, however, did exist between the sexes in the number of large and small chromatin bodies in the nuclei. Male nuclei, in general, had significantly more large chromatin bodies and less small chromatin bodies than female nuclei. However, it was noted that the technique used on the tissue, whether squash or section, was a more important factor in determining the results than the type of tissue under examination.

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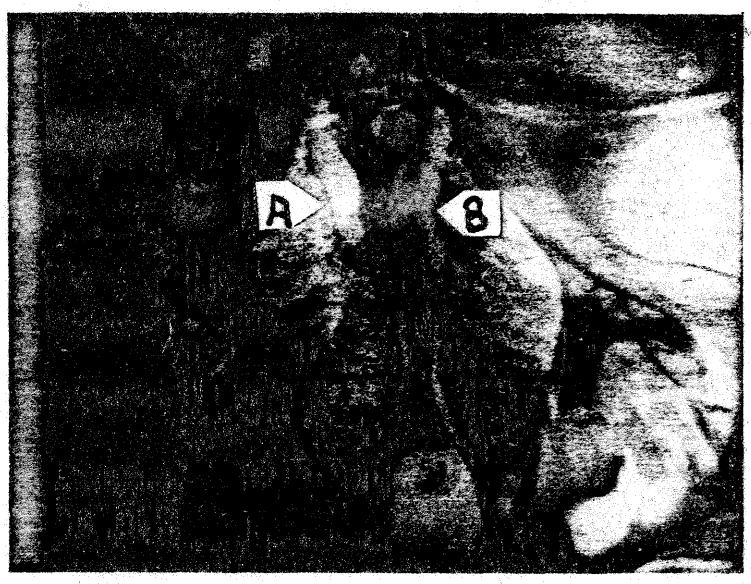


PLATE I Gonads of a male embryo of 16 days incubation

At 16 days of incubation time, the male gonads are of approximately equal size.
A. right testis B. left testis

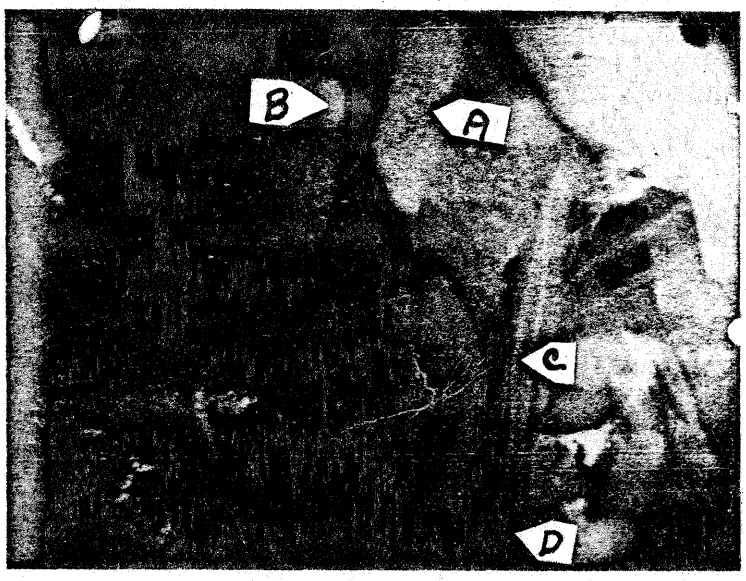


PLATE II Gonads of a female embryo of 16 days incubation

At 16 days of incubation time, the left ovary of the female is much larger than the right. It will serve as the only functional ovary when the hen reaches sexual maturity. The oviduct is well formed at this stage and a shell gland is prominent.
A. left ovary B. right ovary C. oviduct
D. shell gland

APPENDIX B: Summary of raw data tables

Table IA Number of large ($d \geq 0.2$ microns) chromatin bodies touching the nuclear membrane in 100 nuclei of each slide (sl.).

A_1 Duodenal Sections				A_2 Liver Sections				A_3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	95	1	80	2	70	1	60	1	90	4	51
3	113	4	86	5	49	3	56	2	64	7	32
5	96	7	113	7	49	4	67	3	35	8	66
6	110	9	119	8	58	6	58	5	43	9	36
8	112	10	109	10	55	9	64	6	50	10	48
	526		507		281		305		282		233

Table IB Number of large ($d \geq 0.2$ microns) chromatin bodies located near the nuclear membrane in 100 nuclei of each slide (sl.).

B_1 Duodenal Sections				B_2 Liver Sections				B_3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	30	1	25	2	48	1	38	1	47	4	34
3	43	4	45	5	45	3	44	2	30	7	30
5	41	7	31	7	51	4	36	3	29	8	28
6	48	9	33	8	52	6	26	5	35	9	32
8	62	10	44	10	31	9	35	6	21	10	29
	224		178		227		179		162		153

APPENDIX B: continued

Table IC Number of large (d≥ 0.2 microns) chromatin bodies touching and near the nuclear membrane of 100 nuclei of each slide (sl.).

C_1 — Duodenal Sections

sl. no.	♂	sl. no.	♀
2	125	1	105
3	156	4	131
5	137	7	144
6	158	9	152
8	174	10	153
	750		685

C_2 — Liver Sections

sl. no.	♂	sl. no.	♀
2	118	1	98
5	94	3	100
7	100	4	103
8	110	6	84
10	86	9	99
	508		484

C_3 — Liver Squashes

sl. no.	♂	sl. no.	♀
1	137	4	85
2	94	7	62
3	64	8	94
5	78	9	68
6	71	10	77
	444		386

Table ID Number of large (d≥ 0.2 microns) chromatin bodies located in the nucleoplasm of 100 nuclei of each slide (sl.).

D_1 — Duodenal Sections

sl. no.	♂	sl. no.	♀
2	208	1	120
3	262	4	180
5	253	7	210
6	222	9	232
8	262	10	244
	1207		986

D_2 — Liver Sections

sl. no.	♂	sl. no.	♀
2	341	1	306
5	302	3	268
7	316	4	271
8	300	6	278
10	298	9	245
	1557		1368

D_3 — Liver Squashes

sl. no.	♂	sl. no.	♀
1	222	4	261
2	190	7	195
3	227	8	201
5	218	9	261
6	248	10	158
	1105		1076

APPENDIX B: continued

Table IE Total number of all large (d≥0.2 microns) chromatin bodies of 100 nuclei for each slide.(sl.).

A Duodenal Sections				B Liver Sections				C Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	333	1	225	2	459	1	404	1	359	4	346
3	418	4	311	5	396	3	368	2	284	7	257
5	390	7	354	7	416	4	374	3	291	8	295
6	380	9	384	8	410	6	362	5	296	9	329
8	436	10	397	10	384	9	344	6	319	10	225
	1957		1671		2065		1852		1549		1462

Table IIA Average size of large chromatin bodies (d≥0.2 microns) touching and near the nuclear membrane in 100 nuclei of each slide. (sl.).

A_1 Duodenal Sections				A_2 Liver Sections				A_3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	0.33	1	0.35	2	0.28	1	0.25	1	0.24	4	0.27
3	0.36	4	0.38	5	0.25	3	0.27	2	0.26	7	0.21
5	0.35	7	0.29	7	0.31	4	0.23	3	0.26	8	0.24
6	0.34	9	0.34	8	0.28	6	0.25	5	0.25	9	0.23
8	0.31	10	0.37	10	0.25	9	0.26	6	0.26	10	0.24
	1.69		1.73		1.37		1.26		1.27		1.19

APPENDIX B: continued

Table IIB Average size of large chromatin bodies located in the nucleoplasm of 100 nuclei in each slide. (sl.).

B_1 Duodenal Sections				B_2 Liver Sections				B_3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	0.40	1	0.40	2	0.30	1	0.31	1	0.26	4	0.30
3	0.39	4	0.39	5	0.25	3	0.28	2	0.27	7	0.24
5	0.36	7	0.31	7	0.32	4	0.28	3	0.26	8	0.24
6	0.35	9	0.36	8	0.30	6	0.30	5	0.28	9	0.23
8	0.31	10	0.37	10	0.28	9	0.26	6	0.26	10	0.26
	1.81		1.83		1.45		1.43		1.33		1.27

Table IIC Average size of all large chromatin bodies of the nucleus in 100 nuclei of each slide (sl.).

C_1 Duodenal Sections				C_2 Liver Sections				C_3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	0.37	1	0.38	2	0.30	1	0.30	1	0.26	4	0.30
3	0.38	4	0.38	5	0.26	3	0.28	2	0.27	7	0.24
5	0.39	7	0.30	7	0.32	4	0.27	3	0.27	8	0.25
6	0.34	9	0.36	8	0.30	6	0.29	5	0.28	9	0.22
8	0.32	10	0.37	10	0.29	9	0.26	6	0.26	10	0.25
	1.80		1.79		1.46		1.41		1.34		1.26

APPENDIX B: continued

Table IIIA Number of massive chromatin bodies (d≦0.7 microns) both touching and near the nuclear membrane of 100 nuclei in each slide (sl.).

A_1 Duodenal Sections				A_2 Liver Sections				A_3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	5	1	4	2	6	1	2	1	1	4	2
3	11	4	15	5	0	3	1	2	2	7	0
5	10	7	2	7	3	4	1	3	1	8	0
6	11	9	7	8	5	6	2	5	0	9	2
8	11	10	11	10	0	9	2	6	1	10	2
	48		39		14		8		5		6

Table IIIB Number of large chromatin bodies (d≦0.7 microns) located in the nucleoplasm of 100 nuclei in each slide (sl.).

B_1 Duodenal Sections				B_2 Liver Sections				B_3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	12	1	12	2	14	1	20	1	3	4	10
3	19	4	16	5	2	3	2	2	1	7	5
5	15	7	10	7	15	4	18	3	2	8	1
6	15	9	17	8	9	6	14	5	4	9	3
8	10	10	24	10	4	9	4	6	4	10	3
	71		78		44		58		14		22

APPENDIX B: continued

Table IIIC Total number of all massive chromatin bodies ($d \geq 0.7$ microns) of 100 nuclei in each slide(sl.).

C_1 Duodenal Sections				C_2 Liver Sections				C_3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	17	1	15	2	20	1	18	1	4	4	12
3	30	4	31	5	2	3	9	2	3	7	5
5	25	7	12	7	18	4	11	3	3	8	1
6	26	9	24	8	14	6	15	5	4	9	5
8	21	10	35	10	4	9	7	6	5	10	5
	119		117		58		60		19		28

Table IV Number of small chromatin bodies ($d \leq 0.2$ microns) of 100 nuclei in each slide (sl.).

1 Duodenal Sections				2 Liver Sections				3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	391	1	462	2	689	1	667	1	779	4	924
3	478	4	564	5	661	3	792	2	783	7	1059
5	538	7	600	7	746	4	756	3	664	8	892
6	521	9	555	8	686	6	721	5	846	9	1016
8	548	10	586	10	737	9	840	6	803	10	1032
	2476		2767		3519		3776		3875		4932

APPENDIX B: continued

Table V Grand total number of all chromatin bodies of 100 nuclei in each slide (sl.).

1 Duodenal Sections				2 Liver Sections				3 Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	724	1	687	2	1148	1	1071	1	1138	4	1270
3	896	4	875	5	1057	3	1160	2	1067	7	1316
5	928	7	954	7	1162	4	1130	3	955	8	1187
6	901	9	939	8	1096	6	1083	5	1142	9	1345
8	984	10	983	10	1121	9	1184	6	1122	10	1267
	4433		4438		5584		5628		5424		6385

Table VI Frequency of location for the largest chromatin body of 100 nuclei in each slide.

1 Duodenal Sections

males			females		
sl. no.	t/n*	n.*	sl. no.	t/n*	n.*
2	25.3%	74.7%	1	37.7%	62.3%
3	30.0	70.0	4	42.0	58.0
5	32.4	67.6	7	40.4	59.6
6	42.0	58.0	9	33.0	67.0
8	35.0	65.0	10	39.8	60.2
Σ	164.7	335.3		192.9	307.1
Av.	32.94	67.06		38.58	61.42

2 Liver Sections

males			females		
sl. no.	t/n*	n.*	sl. no.	t/n*	n.*
2	16.0%	84.0%	1	15.0%	85.0%
5	24.0	76.0	3	22.0	78.0
7	22.0	78.0	4	21.0	79.0
8	12.0	88.0	6	15.0	85.0
10	18.2	81.8	9	26.3	73.7
Σ	92.2	407.8		99.3	400.7
Av.	18.44	81.56		19.86	80.14

3 Liver Squashes

♂			♀		
sl. no.	t/n*	n*	sl. no.	t/n*	n*
1	34.4%	65.6%	4	19.4%	80.6%
2	31.6	68.4	7	26.8	73.2
3	22.4	77.6	8	31.0	69.0
5	20.9	79.1	9	19.0	81.0
6	19.1	80.9	10	29.1	70.9
Σ	128.4	371.6		125.3	374.7
Av.	25.68	74.32		25.06	74.94

*t/n touching and near
*n in nucleoplasm

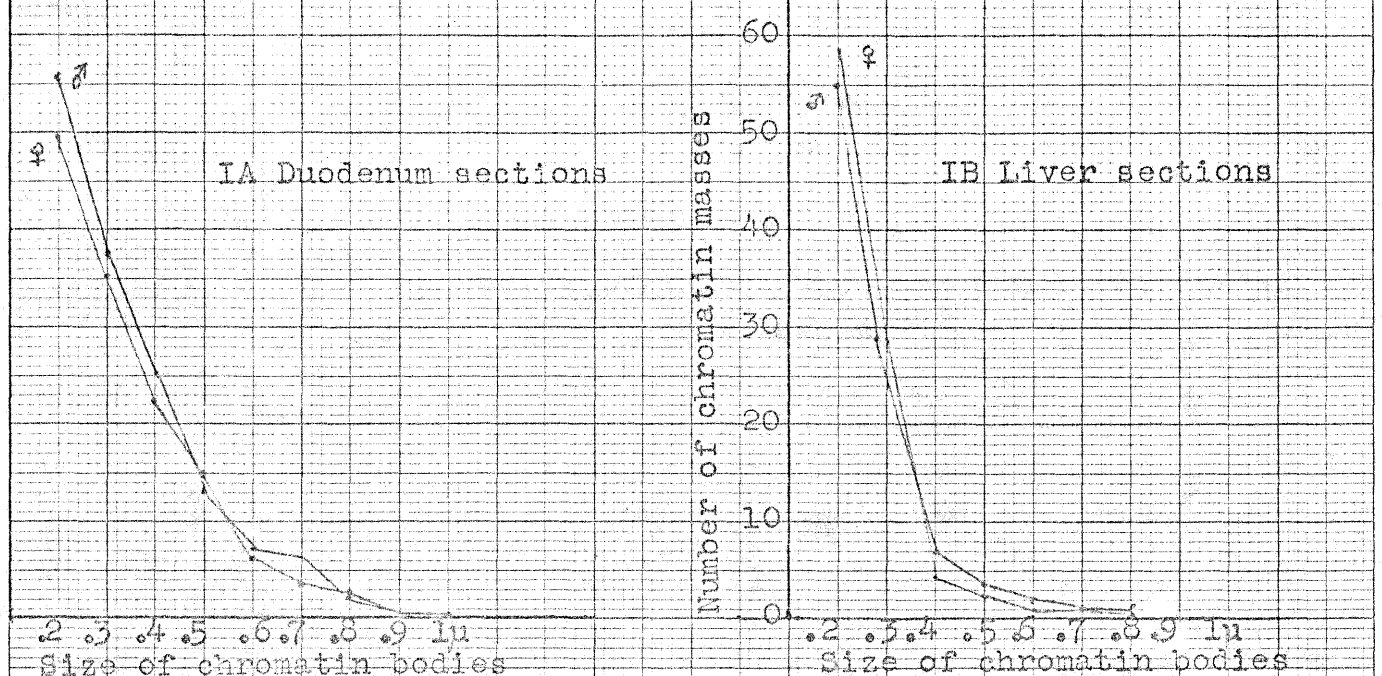
APPENDIX B: continued

Table VII Percent of lightly stained large chromatin bodies (d≥ 0.2 microns) in 100 nuclei of each slide (sl.).

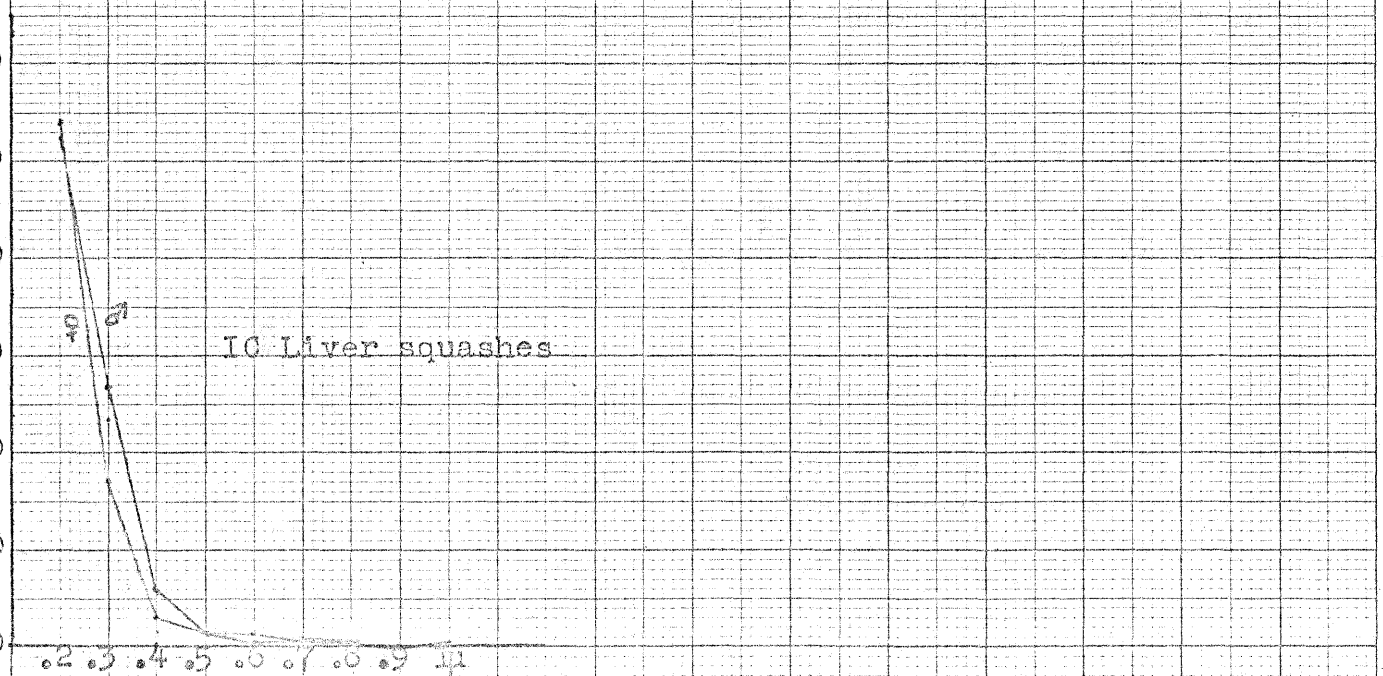
	1			2				3			
	Duodenal Sections			Liver Sections				Liver Squashes			
sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀	sl. no.	♂	sl. no.	♀
2	27.33	1	18.22	2	7.19	1	12.87	1	13.37	4	17.63
3	16.99	4	11.90	5	9.60	3	11.14	2	23.94	7	15.18
5	10.51	7	12.84	7	9.38	4	10.16	3	12.71	8	13.56
6	13.68	9	16.15	8	9.51	6	6.63	5	21.62	9	20.06
8	20.87	10	12.59	10	9.64	9	8.72	6	24.76	10	25.53
Σ	89.38		72.70		45.32		49.52		96.40		91.96
Av.	17.88		14.54		9.06		9.90		19.28		18.39

Appendix C: Frequency distribution graphs of the size of the large chromatin bodies per slide. These represent average values for the five slides of each sex.

I. Graphs A, B, and C are concenred with those large both touching and near the nuclear membrane.

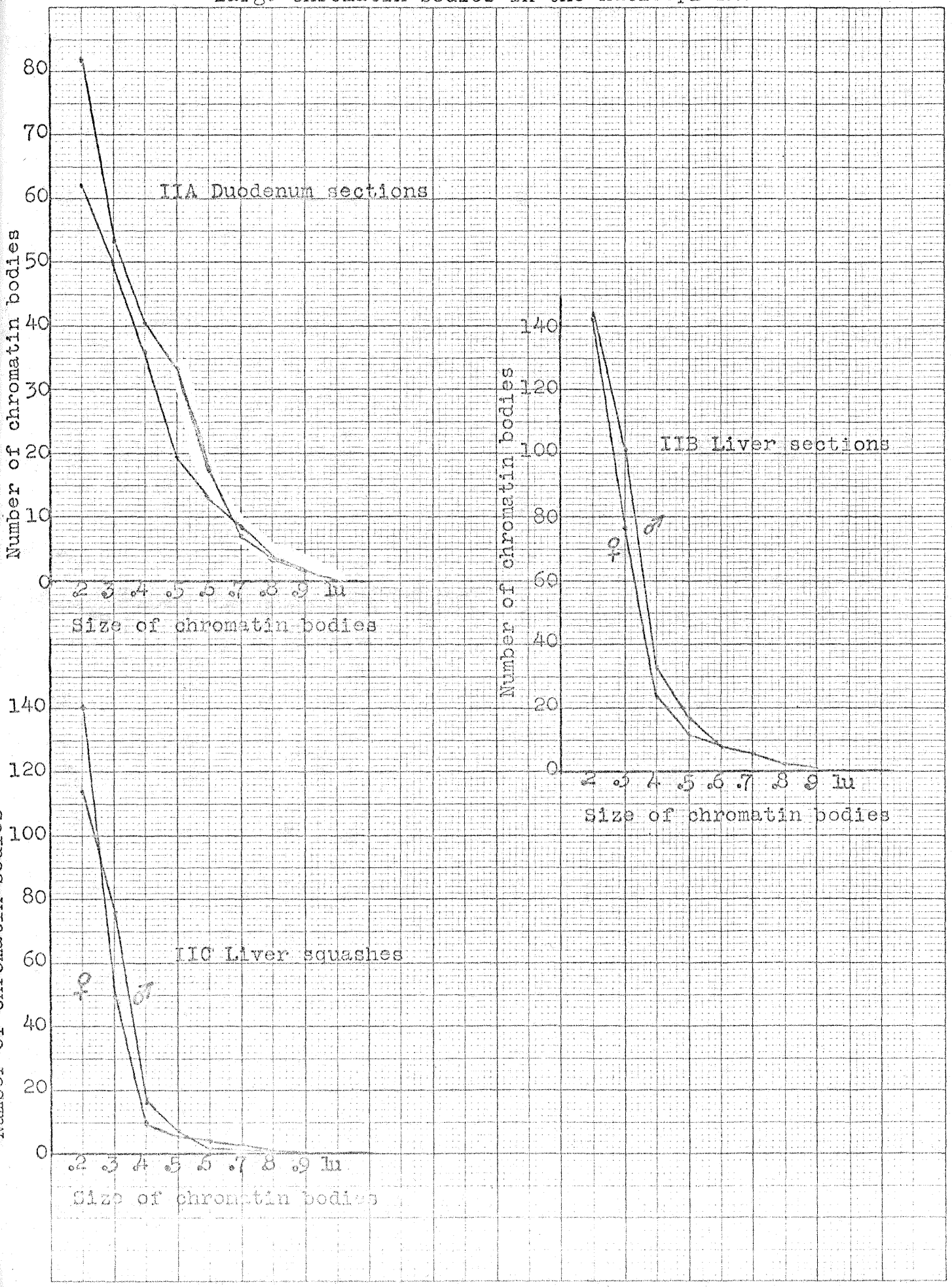


Number of chromatin masses



Appendix C:

II. Graphs A, B, and C are concerned with those large chromatin bodies in the nucleoplasm.



III. Graphs A,B, and C are concerned with all large chromatin bodies regardless of the location.